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BRIEF COMMUNICATION

Influence of peroxides, ascorbate and glutathione on germination and growth in *Lupinus albus* L.

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Abstract

Lupinus albus L. seeds were treated with different concentrations (from 10 μM to 50 mM) of H_2O_2, *m*-chloroperoxybenzoic acid (mCPBA), ascorbate (ASC) and glutathione (GSH). The efficiency as inhibitors on germination and on the subsequent growth of the hypocotyl was mCPBA > GSH > ASC = H_2O_2, which suggest that inhibitory efficiency was dependent on the compound *per se* rather than on its redox nature.

Additional key words: lupine, oxidative stress.

Hydrogen peroxide is an endogenous metabolite commonly produced by many plant tissues (Schopfer 1994). This seems a paradoxical phenomenon bearing in mind the dangerous effects produced by H_2O_2-mediated oxidative stress (see review of Elstner 1982). Nevertheless, plant cells possess an efficient protective mechanism against this stress. The metabolic pathway to destroy H_2O_2 involves reductant compounds such as ascorbate (ASC) and reduced glutathione (GSH), and enzymes such as catalase and peroxidase (Salin 1987, Mittler *et al.* 1991, Asada 1992, Cakmak *et al.*

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Abbreviations: ASC - L-ascorbic acid; GSH - reduced glutathione; mCPBA - *m*-chloroperoxybenzoic acid.
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1993). Toxic oxygen species such as H_2O_2 are generated from mitochondrial oxygen when there is a high rate of oxygen consumption in plant cells (Puntarulo *et al.* 1988). Such conditions arise during seed germination and seedling growth as the respiratory rate increases.

Recently, we have demonstrated that treatment by immersion of derooted lupin hypocotyls in solutions containing peroxides such as H_2O_2 and the xenobiotic *m*-chloroperoxybenzoic acid (mCPBA) and reductants such as ascorbate (ASC) and reduced glutathione (GSH), inhibited growth and rooting, the effect being dependent of the compound *per se* rather than on its redox nature (Cano *et al.* 1996). In the present work, the influence of the redox state on plant morphogenesis is studied with a new approach, treatment to seeds instead to hypocotyls, to investigate the influence of these compounds on the seed germination and on the subsequent growth of the intact lupin hypocotyls.

Seeds of *Lupinus albus* L. (cv. Multolupa), were imbibed (24 seeds in 100 cm^3) for 24 h in water (control) or aqueous solutions (from 10 μM to 50 mM) of H_2O_2, mCPBA, ASC or GSH, and grown in damp vermiculite at 25 °C and darkness. The percentage of germination was noted at two days after planting. No further germination was observed irrespective of the treatment. Hypocotyl growth was followed for 16 to 19 d after planting by measuring the length of the hypocotyls at 1 to 3 d intervals. Commercial mCPBA (82 % purity) was purified as described previously by Acosta *et al.* 1993. All the test solutions were stable in the assay conditions for more that 24 h (Cano *et al.* 1996). Experiment were repeated at least three times. Data presented correspond to a representative repetition.

Table 1. Influence of ASC, GSH, H_2O_2 and mCPBA on hypocotyl growth. The length of hypocotyls at the end of the growth period (15 - 18 d) is expressed as percentage of control values (*i.e.* final length of hypocotyls from water imbibed seeds). Means ± SE of 8 to 24 plants (depending on the germination percentage).

Concentration [μM]	ASC	GSH	H_2O_2	mCPBA
10	96 ± 4	102 ± 5	98 ± 3	94 ± 12
50	104 ± 3	-	99 ± 2	-
100	-	94 ± 4	-	-
200	98 ± 2	-	104 ± 2	106 ± 2
1000	92 ± 3	98 ± 5	94 ± 3	51 ± 5
10000	83 ± 2	73 ± 5	78 ± 4	0
50000	69 ± 2	50 ± 7	54 ± 2	-

When seeds were imbibed with the different test solutions, no effect on germination was observed except at the highest concentrations of GSH (50 mM) and mCPBA (10 mM), which reduced germination to 33 and 0 %, respectively, compared to the control (100 % germination in water-imbibed seeds). The influence on hypocotyl growth in germinated seeds was dependent on the concentration as well as on the type of compound. No significant effect on the final growth of hypocotyls (Table 1) or their growth kinetics (Fig. 1) was observed when seeds were treated

with H_2O_2, ASC or GSH at concentrations ranging from 10 μM to 1 mM. Growth was inhibited at 10 mM and even more when the concentration of these compounds was increased to 50 mM. Compared with the growth of the control (100 %), the reduction in growth was 46, 50 and 31 % for 50 mM H_2O_2, GSH and ASC, respectively (Table 1). The xenobiotic mCPBA was the most effective in growth inhibition (Fig. 1). Thus, 1 mM mCPBA inhibited hypocotyl growth by 49 % (Table 1), a percentage that was similar to that obtained with a concentration 50 times higher of H_2O_2. The growth kinetics (Fig. 1) show that the reduction in growth produced by the different compounds was due to an increase in the duration of the initial period of low growth rate (up to 4 - 5 d in control), as well as to a decreased growth rate during the linear growth period (from 5 to 12 d age in control). Both effects were magnified when the concentration of the compounds increased.

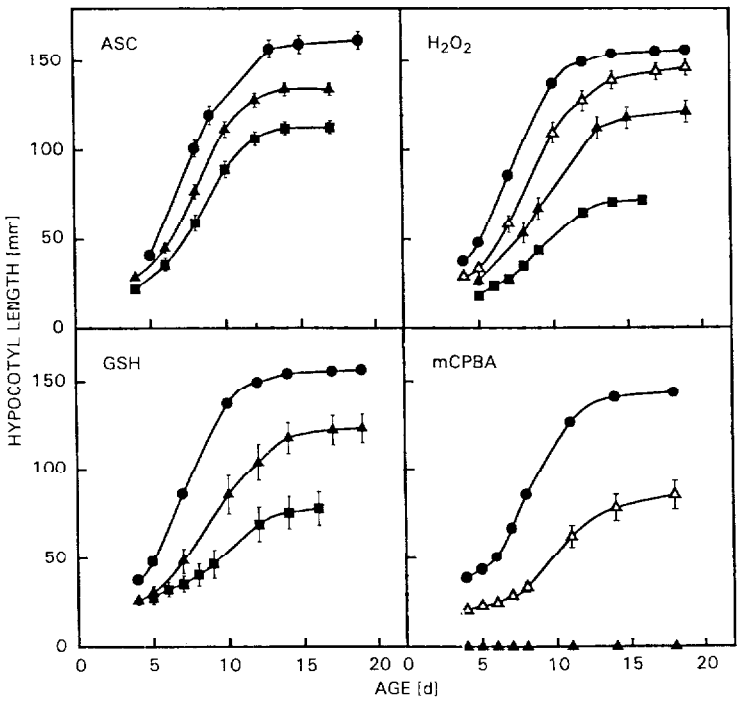

Fig. 1. Growth kinetics of hypocotyls from treated seeds. Seeds were imbibed for 24 h in water (control, *closed circles*) or aqueous solutions containing ASC, H_2O_2, GSH and mCPBA at different concentrations (1 mM - *open triangles*; 10 mM - *closed triangles*; 50 mM - *closed squares*). The length of hypocotyls was measured at different age. Mean values of 24 (or 8 in the 10 mM GSH treatment) plants. *Bars* denote SE when larger than symbols. Data corresponding to concentrations lower than those represented are omitted for clarity since they were very close to control.

Hypocotyls from ASC- and mCPBA-treated seeds showed no visual damage at any concentration. GSH (50 mM) as well as H_2O_2 (10 and 50 mM) produced brown spots along the hypocotyls.

Taking into account the smallest concentration that produced a significant

reduction in the processes studied (Table 1), the assayed compounds can be classified as inhibitors of both processes (germination and growth) in the following order: mCPBA > GSH > ASC = H_2O_2. This order coincides with that recently obtained for the growth and rooting of derooted lupin hypocotyls when the hypocotyls were directly treated with these compounds and support the hypothesis proposed in our previous paper (Cano *et al.* 1996) that the inhibition power appears to be inversely related with the capacity of plant tissues to detoxify the different compounds. Thus, in the case of peroxides, the higher the capacity of the detoxifying machinery (mainly peroxidase enzymes), the lower the toxicity of the peroxide. In fact, mCPBA was 250 times as effective as H_2O_2 in inactivating peroxidase (Acosta *et al.* 1993), which implies that the peroxide-detoxifying mechanisms are less effective in mCPBA than in H_2O_2-treated plants. Present results also confirm that the efficiency to inhibit germination and growth was dependent on the compound per se rather than on their redox nature. Thus, reductant compounds such as GSH assumed to play a protective role against oxidative stress due to their involvement in the peroxide-detoxicant route catalysed by ASC-peroxidase (Asada 1992), exhibited high capacity to inhibit growth (Table 1). Indeed, several toxic species such as free radicals of glutathione and glutathione hydroperoxide can be generated from GSH in the presence of peroxidase (Harman *et al.* 1986, Madeiros *et al.* 1987), which might account for the high toxicity of GSH observed here.

The hypocotyl growth was more sensitive than seed germination to the inhibitory effects of the compounds (Table 1). Bearing in mind that the concentrations of the different compounds were presumably higher during the early phase of germination, the lower sensitivity of germination to all the assayed compounds suggests that the capacity to detoxify these compounds was higher during the first phase (embryo growth) than during the subsequent phase (hypocotyl growth). This is especially evident for H_2O_2 and ASC, which permitted 100 % germination at any concentration in spite of the significant growth inhibition produced by concentrations higher than 1 mM (Table 1). The above is in agreement with results showing that the activity of the enzymes (such as catalase, the H_2O_2-scavenging ASC-peroxidase, and those related with ASC regeneration) active against oxidative stress increased during seed germination (Puntarulo *et al.* 1988, Cakmak *et al.* 1993, Mullen and Gifford 1995). In addition, the growth inhibition produced by mCPBA and GSH was lower in younger that in older lupin hypocotyls (Cano *et al.* 1996). Furthermore, the evolution of the longitudinal distribution of guaiacol-peroxidase activity in lupin hypocotyls (Sánchez-Bravo and Núñez 1990) was parallel to that of the growth rate (Ortuño *et al.* 1988). All the above evidence points to the existence of a changing detoxifying capacity during growth, the young cells having a more efficient protective mechanism that the older tissues.

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